iHELMET: A 3D-printing solution for safe endoscopic Ca2+ recording in social neuroscience

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Methods Manuscript

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Abstract

Background: In vivo calcium imaging using a microendoscope is a state-of-the-art technique to study the cellular activity inside the brain of freely moving animals such as mice or rats. A problem that can arise in social behaviour tests in rats, or similar size rodents, is that one animal interferes with or may even damage the miniature endoscopic camera attached to the second animal.

New method: We outline an inexpensive, lightweight, 3D-printed protector (iHELMET) that surrounds but is not in physical contact with the camera, together with details of its design and construction.

Results: Using a simple design, we demonstrate successful protection of the endoscope and recording in a social situation such as the social dominance tube test.

Comparison with existing methods: The helmet's 3D-printed dimensions can be readily adjusted work with various micro-endoscopes, which may be more difficult for the only other system of which we are aware.

Conclusions: In addition to camera protection, features of the design aid camera stability, helping to secure more optimal imaging of calcium transients in specific regions of interest during long recording sessions.

Introduction

Growing interest in the neurobiology of social behaviour has led to the use of optical calcium imaging as an indicator of neural activity in various brain regions. It is paramount that this is done in behaving animals that are freely interacting with each other. In calcium (Ca^{2+}) imaging, a genetically encoded calcium indicator (such as the GCaMP6 series - (Chen et al., 2013) binds with Ca^{2+} ions and reports their presence as a rapid increase in the intensity of fluorescence emissions. As the intracellular Ca^{2+} concentration decreases, the fluorescence also gradually declines (Nakai et al., 2001; Tallini et al., 2006). This sequence of events is defined as a Ca^{2+} spike or Ca^{2+} transient. As these transients are observed repeatedly in specific locations, regions of interest (ROIs) can be identified in which specific transients are observed. The implication is that such Ca^{2+} transients are likely to be from individual neurons.

The neural correlates of social interaction cannot easily be studied using 2-photon-imaging in head-fixed animals on track-balls, although limited facets of social behaviour in virtual-reality are becoming available (Stowers et al., 2017). The recently developed method of choice is the use of lightweight miniature endoscopic cameras (~2gm) which, when coupled to genetic expression of Ca^{2+} reporters such as GCaMP6 constructs (either via viral vectors or transgenic animals) and an implanted GRIN lens targeting the specific intracerebral ROI (Ziv et al., 2013), provide images of Ca^{2+}-transients in real time. With the GCaMP6 expressing virus construct, implanted lens and attached camera, it is possible to image the precise brain location and time-course of such transients (Ghosh et al., 2011; Jercog et al., 2016; Figure 1A). A key value of this approach is the ability to examine large numbers of "cells" simultaneously and use both single-cell and ensemble analyses.

In social behaviour, such as in the tube test of social dominance, a fundamental problem is that there may be physical interference by one animal with the camera on the other animal from which recordings are being taken. This interference may only be momentary, such as a brief paw movement, or may involve recurrent somatosensory interactions between animals or even brief mildly aggressive attacks. These can be sufficient to disrupt the stability of continuous recording.

An additional type of interference may be introduced because of the confined space of the narrow tube used in the tube-test of social dominance. The diameter of the tube has to be wide enough to let each animal to walk through it easily, but should be narrow enough that the two animals cannot pass each other. This condition of confined space in the tube test introduces a set of new problems for stable Ca^{2+} recordings. Specifically, when entering the tube, the head mounted endoscope risks becoming caught at the entrance, and even when in the tube, sideways head-movements result in the camera being pushed or even banged against the walls of the tube. Such interference has apparently not yet been a problem for studies in smaller animals such as mice (Kingsbury et al., 2019), but our experience of working with animals that are approximately ten times larger and physically much stronger indicates that it is a major problem. During an ongoing social neuroscience project, we observed good stable imaging when the rats were not interacting with each other (Figure 1A). Social interactions may cause minor interference that can ordinarily be compensated by motion-correction software, but can increase to a level that is difficult or impossible to correct in this way (Figure 1B,C). Additional problems can include "ghosting" of cell outlines from one point in a recording session to another due to camera motion, and there can be a a momentary shut-down of imaging after a robust bang of the camera against the side-walls. Such interference is undesirable given the clear cost in both workload and economic terms. Given that new gene editing techniques will likely soon enable more studies to be done on rats rather than mice (Hsu et al., 2014; Till et al., 2015; Zhang et al., 2014), it may be of interest to report how we solved these problems.

One solution is to create barriers between the interacting animals as in the classic 3-chamber sociability / social novelty test (Crawley, 2004; Moy et al., 2004). The two animals are physically separated by a permeable barrier through which they can see, smell and hear each other, but somatosensory contact is limited. In the 3-chamber task, the 'test' animal in the larger space shows social interactions with the 'enclosed' animal(s), but these interactions, including whisker contact, are limited. Neurophysiological and optical recordings have nonetheless been made of such social interactions using this and similar tasks (Liang et al., 2018; Murugan et al., 2017).

We therefore considered an alternative approach that permits physical contact between the animals by developing a lightweight 3D-printed helmet with suitable dimensions and rigidity for successful endoscopic recording. The helmet is placed on the animal daily and held rigidly to a separate 3D printed baseplate-surround cemented onto the animal. We have successfully used this system in various social situations, including the social dominance tube test.

Materials and Methods

3D-designing and Printing: The iHELMET was designed using Fusion 360 a 3D modelling software. It is made using PLA (PolyLactic Acid) on an inexpensive Replicator 2 Desktop 3D printer (https://www.makerbot.com/3d-printers/; Figure 2A). Ready to print .stl files are available in the attached Supplementary information. The dimensions of the plastic were determined to be, internally, no more than 2 mm larger in the x, y plane than those of the Inscopix endoscopic camera, but are easily adapted for other types and makes. The helmet is so light that it adds only 4.0 g to the typical <2.0 g of the nVISTA camera of an Inscopix system (roughly <2% of the weight of the rat). Similar considerations apply to the Doric and open-source UCLA recording systems (http://doriclenses.com/life-sciences/307-miniaturized-fluorescence-microscopy; https://www.inscopix.com; http://miniscope.org/). Including the width of the plastic and design considerations, this resulted in a final unit that was 15 mm x 28 mm (x, y), and a height of 56 mm. The costs of construction of each unit are modest, with the asset costs of the 3D Printer shared between several lab groups. In-house construction enabled us to make regular modifications of the design as the project unfolded. Once printed, these parts were dry-fit tested before using.

Insert Figure 1 about here

Camera: The Inscopix endoscope which we used (Figure 2B) has external dimensions of 11mm x 14mm x 20 mm and weighs 2 gm. On the animal, the 'miniscope' is normally placed into to a "baseplate" made of metal or hard plastic (with various designs in different systems), that secures the camera rigidly in the same place on each session above a miniature implanted GRIN lens (e.g. 1 mm diameter) that extends to the brain area of

interest in which GCamp6f is expressed. Details of the principles behind *in vivo* endoscopic imaging are readily available at the above listed websites.

Experimental subjects*:* The focus of this technical note is on the helmet design but a brief comment about the experimental subjects is required. We used male Long Evans hooded rats (Charles River), typically weighing 470 - 600 g. The animals were typically housed in groups of 2 rats together in a social cage, with suitable protection of the GRIN lens as described below. They were maintained on *ad libitum* food and water and the study conducted under the auspices of the laboratory's UK Home Office Project and Personal Licences for animal research.

Surgery, Baseplate and Baseplate-Surround: The first step in the use of these animals is the microinfusion of GCamp6f virus under isofluorane anaesthesia at a specific intracerebral target (the prelimbic region of the prefrontal cortex). Approximately 3 weeks later, allowing time for the virus to express, a GRIN lens is implanted under anaesthesia in a second operative stage, with full recovery thereafter. This lens is secured using a combination of skull screws, Super-bond C&B (Sun Medical Co. Ltd, Japan), and dental cement to provide firm anchoring. The miniscope requires a "baseplate" (5 mm $\times$ 5.5 mm for INSCOPIX, a different size for other suppliers) which incorporates anchoring magnets and a side-mounted screw. The magnets hold the miniscope on the head of the animal in a consistent position to retrieve the same field of view across imaging sessions. The baseplate is cemented into place on the skull of a laboratory rat under anaesthetic, being positioned while imaging with the camera to optimise the field of view of cells in which the virus is expressing.

A key feature of our innovation arises from the creation of a "Baseplate-Surround" (Figure 2C). Specifically, the daily anchoring of the helmet requires a separate 3D-printed PLA Baseplate-Surround whose function is to hold the helmet rigidly (shown surrounding the metal baseplate in Figure 2C(i and iii)). The has its own dedicated protector cap used when the animal returns to its home cage and removed at the start of a recording session (Figure 2C(ii)). Its purpose is to protect the GRIN lens and baseplate whilst the animals are in their social groups.

When implanting the baseplate surround, it must be aligned with the baseplate such that, when the endoscope is placed into it and the iHELMET then attached, the helmet will not be not in direct physical contact with the endoscope. A side-view of the baseplate-surround in Figure 2C (i) bottom. Aligning the front screw-holes of the baseplate-surround to that of the baseplate screw works well, together with keeping the baseplate-surround as far back as possible in order to leave around 1-1.5 mm space between it and the baseplate (Figure 2C (iii)). After positioning the iHELMET over the endoscope, it typically requires a few mm of forward movement to bring it to its final position (see Supplementary Video 3_procedure for the complete instructions). A design feature is that, as the baseplate-surround can limit the access to the baseplate screw, we drill out the adjacent part of the baseplate-surround to secure optimum access (Figure 2C(iv)).

A locking nut (A2 M1.6) is embedded in the baseplate-surround and the helmet equipped with an A2 M1.6X5 bolt (https://www.screwsandmore.de/de; Figure 2E). This combination of nut and bolt is suitable for the maximum torque faced by the iHELMET in the tube-test when one animal pushes on the iHELMET of the other animal with any force or while animals helmet is caught at the entry of the. This choice of screw-thread has worked flawlessly in our experiments, but other sizes of screws may be preferred in different laboratories.

Attaching the iHELMET and recording cable: After the baseplate-surround protector cap is removed on the animal, the endoscope is first attached to its baseplate, and then the helmet slid down the recording cable to lock onto the baseplate-surround and secured using the helmet screw. The lightweight cable connects the endoscope to an image capture system (DAQ), or an image illumination system in the case of DORIC, sometimes using a ceiling-mounted commutator to enable freedom of rotational movements by the animal. The cable is highly flexible, but there are two additional refinements to the iHELMET which emerged from its early use. One refinement is to protect the cable, the other to prevent sudden changes in cable tension affecting the camera.

The first was achieved using a short section (15-20 cm) of cable sheathing (resistant to rodent teeth) around only the lower part of the cable. This sheathing is commercially

available as "split cable-sheath". We used a "wire-loom" tool to put this split sheath onto the cable, with the hollow part of the loom containing the cable and the solid part used to split the sheathing. Forward motion of the loom while holding the sheath, resulted in the sheathing being easily applied over the lower part cable (See Supplementary (Wire_Loom.stl file) to enable users to 3D print the tool). The vertical extent of the protective sheathing can be quite short as it serves to guard against any grabbing by the other animal.

The second refinement was to ensure that any change in cable tension is transmitted only to the helmet but not the camera. This was achieved by attaching the cable inside its cable-sheath to a rigid rear-mounted vertical post of the iHELMET using a cable clip (Figure 2D and E; black in colour). It can be squeezed gently (to grip both the cable and cable sheath) and then secured to the helmet. This ensures that any change in cable tension is transmitted only to the helmet but not the camera.

Controlling movement artefacts: A separate benefit of using this particular design of helmet is improved limitation of movement artefacts. While normally the camera is held well within its baseplate, it may nonetheless be subject to very small movements (fractions of a millimetre) in both the x, y and separately the z axis, typically caused by any changes in the tension of a free-to-move cable. These may minutely change the field of view, enough to cause image instability, which may or may not be correctable by image stabilisation software. It is clearly desirable to keep these artefacts to a minimum.

The final arrangement of the endoscopic camera in its helmet is shown in Figure 2F. Dummy cameras were also used so that, when recording from only one animal, the second animal in the social encounter had a similar helmet arrangement on its head.

Results

The results to be presented are in three stages. The *first* stage describes the problems we faced when we first attempted to use the endoscopes in a social situation, including the

movement artifacts experienced (Figure 1). The *second* describes our successful design, construction and use of the iHELMET (Figure 2). The *third* presents data relevant to limitation of movement artefacts. Steps 1 and 2 are qualitative, whereas Step 3 is quantitative (Figure 3).

The problem of movement artefacts: Figure 1A shows the typical arrangement for the miniature endoscope and a field of view upon which brief transients are visible as the animal moves around. In our attempts to use this standard arrangement in the tube-test, we ran into the problem that the camera could hit against the slit that had been cut into the plexiglass tube or be subject to interference from the other animal in the encounter (Figure 1B). We were nonetheless able to run a number of sessions from 4 animals before discontinuing due to concern about potential damage to the endoscope and its recording cable (Supplementary Video 1 without helmet). To quantify the extent of the movement artefact problems which arose, we computed Pearson correlations between corresponding pixels of successive video frames. In a condition of stability, the correlation coefficient (r) should be at or very close to 1.0. However, as shown in a representative set of data in Figure 1C, the near unity value of the correlation was subject to occasional 'jolts' that coincided with the problems we were observing. We defined motion artefacts arbitrarily as any reduction of the r value below 0.75.

The iHELMET solution; design, construction and use: The design of an effective 3D printed helmet evolved through numerous design stages intended to solve key problems. The design requirements were:

- Removable and replaceable easily on a daily basis.
- Connecting rigidly to the animal in manner that surrounds but does not interfere with the camera or the base-plate holding it.
- The inner dimensions of the helmet should leave a physical gap of at least 1 mm between it and the camera, but the entire construction should still be narrow, suitable for the tube test.

- The helmet surround should not result in excessive heating of the endoscope, allowing free movement of air around it.
- The helmet should include a rigid posterior-mounted post to which the recording cable can be attached, helping to ensure the safety of the thin wires within the cable that carry power and the optical signal. In such an arrangement, any 'jerky' movements of the cable would not be transmitted beyond the helmet to the camera.
- The baseplate-surround should have a separate small 3D-printed plastic protector that can be placed after a day's recording to protect the integrity of the GRIN lens in the home cages that may house several animals.
- In cases in which imaging is done from one of two animals interacting socially, a "dummy" camera system should be installed on the test-mate from whom recordings are not being taken. This is intended to prevent technique-induced bias with respect to social interactions between animals.
- 'Retro-fittable' to the existing design features of commercial or open-source systems being used (likely requiring small 3D-printing differences for different systems).

Insert Figure 2 about here

Figure 2 shows the various components of the final iHELMET design. The outline of the construction in Materials and Methods (above) will be seen to meet each of these conceptual requirements. In particular, we have not observed any damage to either the camera or the cable since the introduction of this iHELMET system.

Positioning the helmet on the animal: When an animal is removed from his home cage for a daily recording session, the first step is to remove the protective cap of the baseplate-surround. This exposes the 'clip' arrangement of the baseplate-surround to which the helmet attaches. The endoscope and its attached cable are then moved into position, using in the case of the Inscopix system, the 4 magnets for effective positioning in the baseplate followed by tightening of the side-mounted locking screw. The helmet is then moved down the cable and screwed into position on the baseplate-surround. The experimenter checks

that its positioning results in no physical contact between the helmet and the camera and then attaches the power/recording cable using the cable clip. Once used to these steps, the experimenter can normally do these successfully in 10-30 sec. Figure 2F shows a cartoon of the helmet positioned on a rat's head before entering a social situation. Either both rats will have a camera, or one will have a real camera and the other a dummy camera (Supplementary Video 2).

The benefits; limiting unexpected movement: The third facet of our results is whether such an arrangement is successful in both protecting the camera and reducing movement artefacts and enabling better motion-free recording. The temporal characteristics of Ca^{2+}-transients is a sharp rise and generally slower falling phase of a Ca^{2+}-signal. These are often plotted as a time-stamped raster with time on the x-axis once criteria for time-stamping are identified (amplitude-threshold, rise-time, fall-time etc.). These signals may then be time-locked to separately recorded behavioural signals manually, or via software such as DeepLabCut (Mathis et al., 2018; Nath et al., 2018) and the secondary analysis then undertaken examining correlations between physiological and behavioural measures. Clearly this behaviour/physiology correlation requires the imaging to be stable.

Insert Figure 3 about here

Unavoidable image motion that occurs in the nature of *in-vivo* recordings can be compensated to some extent by the 'motion correction' feature available in calcium image analysis software (such as the Inscopix Data Processing Software - IDPS). However, motion artefacts often correspond to a change in the imaging plane, rendering motion irreconcilable. A key benefit of our helmet was a striking reduction in the mean number of motion artefacts detected during imaging with iHELMET compared to without iHELMET (Figure 3B and 3C). As in Figure 1, the Pearson's correlation was calculated between successive frame images from the spatially band-passed recordings for eight animals (4 with iHELMET, 4 without iHELMET). Motion artefacts were identified as a correlation $r < 0.75$ (shown as a dotted line in Figure 3B) and the total number of artefacts for each animal numerically corrected to account for recordings going first out and back into focus (dividing by 2).

An important qualification we should address is whether there are aspects of behaviour that are changed by the use of the iHELMET. Qualitatively, and mindful of the low weight of the device, there appeared to be no obvious differences. One quantitative check we made, however, was to examine social dominance in the tube test between 8 pairs of animals under conditions in which they were tested without a helmet or separately with the helmet. Supplementary Figure 1 shows the striking correlation between these two conditions and a correlation coefficient of r = 0.92.

Discussion

The primary aim of creating a 3D-printed helmet was to protect the delicate endoscopic camera. During development of the design through various stages, the iHELMET design evolved into a constellation of three component parts. The helmet itself, the cable clip that restricts the changes in torque introduced by cable inertia reaching the endoscope, and the baseplate surround that is implanted to surround the baseplate and which holds the helmet to the animal. The outlined design fulfils each of several design considerations. An added benefit was that, by anchoring the recording cable, there can be improved stability of Ca^{2+} imaging. In our experience, it enables long periods of safe, stable recordings from animals tested in social situations over long daily sessions, and several weeks or more.

Endoscopic Ca^{2+} imaging has rapidly become a method of choice for examining diverse issues - place cell stability in hippocampus (Ziv et al., 2013), time-stamping of memory ensembles (Rubin et al., 2015), contextual linking as a function of temporal proximity (Cai et al., 2016), and aspects of memory consolidation (Attardo et al., 2018). Social behaviour, however, presents a challenge. Unprotected endoscopic imaging can be successful (Liang et al., 2018; Murugan et al., 2017), but difficulties can arise. The solution proposed here is ideal for an endoscope camera for use with larger rodents such as rats, but other laboratories facing similar issues have proposed alternative methods. For example, a protective cone to protect a tetrode micro-drive for extracellular single-cell recording has

been described (Nguyen et al., 2009), and others have also outlined a calcium imaging protection system (van den Boom and Bos, 2018) (https://miniscope.org/index.php/Miniscope_Baseplate_and_Protective_Cone_for_Rats). While ingenious and suitable for mice, there are some restrictions to the van den Boom and Bos (2018) design. There is incompatibility with existing calcium imaging systems as it requires construction of a new, carefully milled, aluminium camera baseplate to which the protective cone fits. This then has consequences for the imaging. In their own words *"having a distinct baseplate design can increase the distance between the endoscope and GRIN lens, requiring the use of a relay lens".* There appears also to be no provision to nullify the impact of sudden cable movements. The present helmet design is compatible with existing miniscope systems, but we advise laboratories to check the dimensions of the miniscope they are using and edit the provided iHelmet and baseplate-surround files (.STL) to compatible dimensions.

Beyond protection, a secondary but no less important facet of the design is its ability to limit motion artefacts when imaging. Having stable signals throughout the course of a recording session is essential and a natural feature of conventional tetrode recording but harder to realise with endoscopic recording. Using the iHELMET , the number of motion artefacts detected was significantly reduced, with minor motion observed in certain constrained and socially interactive situations. The use of the helmet may, therefore, even be desirable in situations in which only a single animal is being tested - where there is a risk of an unprotected camera hitting against some feature of the apparatus or in a large apparatus in which there may be considerable cable movement. Furthermore, the helmet can only complement other protective measures during social interaction (e.g. physical barriers).

We have now used the helmet in two separate social testing situations. One was a modified 3-compartment sociability/social novelty test. The animal from which imaging was taking place was free to move around a large test arena containing two small enclosures. The target animals were contained within these enclosures, but a design fault intended to increase the possibility of sensorimotor interaction was to make the enclosures in such a way as to permit the target animals inside to be able to reach a paw outside and so contact the recording animal. On one occasion, this led to unintended gripping of the recording

cable and consequent damage. The new arrangement with the cable sheath and helmet clip completely prevents this type of accident without the need for any greater sensorimotor barrier between the animals. Both animals can still reach each other without danger. The second testing situation was the social dominance tube-test in which two rats meet in a tube from which the subordinate one retreats or is forced to retreat, and the dominant animal pushes or follows the subordinate. In our laboratory, the clear acrylic tube has a small 2 cm 'slit' along its long top surface wide enough for the camera and its associated recording cable, but also wide enough for a paw to reach through. In this situation, we had previously observed both frequent physical contact between the camera and one side of the slit (causing interruptions of successful imaging), and between the paw of one animal and the endoscopic camera on the other. Again, the design solves both problems. Even if the helmet makes contact or even knocks the side-walls of the tube, the camera is safely inside making no physical contact with it. Similarly, paw movements against the sides of the helmet are of no consequence.

The recordings shown in this study were from the medial prefrontal cortex (mPFC). The stereotaxic coordinates were 3.2 mm rostral from bregma and 0.8 mm lateral from the midline. The iHELMET can, in principle, be used while recording from any brain region, but prospective users should consider the possibility of the helmet interfering with the normal body movement or the behaviour of the animal. For example, recording from the olfactory bulb may be a challenge but likely not insuperable. Our tests of social dominance between 8 pairs of co-housed animals revealed a high *correlation* (r = 0.92) in tests with and without the helmet.

The helmet may be said to "*..kill two birds with one stone*" as the design successfully addresses two practical problems in endoscopic recording - camera protection and reducing movement artefact. It is especially suited for stronger freely-moving animals such as rats.

Credit

Richard Morris: Conceptualization, Supervision, Writing.

Kapil Saxena, Patrick Spooner: Methodology, Construction, Figures, Experimentation.
Kapil Saxena, Rufus Mitchell-Heggs: Software, data-analysis, Visualization, Figures, Reviewing and Editing.

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Declaration of Competing Interest

The authors declare no conflicts of interest, financial or otherwise. The iHELMET system was developed in the laboratory of Prof. Richard Morris at The University of Edinburgh and is not patented. The 3D printing files are free to edit as required. We would welcome citation of this article if used.

Figure Legends

Figure 1: ***The problem: situations that emerged during a calcium imaging session in the tube-test.*** **A)** The head-mounted endoscope as designed to be used, a representative field of view with exemplar $\Delta F/F$ measures of Ca^{2+} transients secured during recording. **B)** Two animals engaged in a contest in the tube-test of social dominance (top and side views). The acrylic tube has a 2 cm slit allowing the endoscope to move freely along its length. The unprotected scope is clearly visible in the animal on the left. The confined space puts the camera in danger of being pushed against the side-walls of the slit or of being damaged by the other animal. **C)** Pearson correlations were computed across pixels between adjacent frames (0.05 s apart) throughout a representative 50 s session.

Figure 2: ***The solution: design and construction of the iHELMET.*** **A**) A side- and partly rotated view of the final design of the 3D-printed iHELMET. Note connecting clips to the baseplate-surround at the bottom, window panels to allow free air movement, and rear post for connecting the recording cable. **B**) Photo of a typical miniature endoscope weighing < 2 gm and associated baseplate with four miniature anchoring magnets (Inscopix). **C**) Top and side view of 3D-printed baseplate-surround unit. (Ci): Side view shows holes to enable more secure anchoring with skull cement; (Cii): baseplate-protecting-cap in place (for when animals are in their home cages); (Ciii): Image of baseplate-surround on a rat's head while surgical implantation was being carried out, the green part at the centre is a small baseplate cover that protects the lens. Notice the baseplate stainless steel screw; (Civ): A notch is drilled out in the baseplate surround at the time of implantation to ease the access to the baseplate screw. **D**) Recording cable with miniature endoscope and clip, front and side-view of iHELMET, and cable grip system. **E**) Final view of the helmet with cable secured for recording. **F)** Carton showing helmet on a rat with all of the essential components.

Figure 3: ***The use: reduction in movement artefact when using the iHELMET.*** **A)** Views of animals wearing helmets during the pilot studies of its use (side and top views). Note that even when the two helmets are in contact, that contact is not transmitted to the camera inside, nor when a helmet hits against the slit in the acrylic tube used for the tube-test. **B)**

Pearson correlation between pixels of adjacent frames over a representative period of 50 s recording. Cartoons display without and with-helmet conditions. Note stability (red) of the recording condition with the helmet. **C)** Display of mean and number of individual movement artefacts, these being arbitrarily defined as situations in which the frame-to-frame correlation fell below 0.75. Note striking reduction in the with-helmet condition. **D)** Representative FOV showing ROIs identified using CNMFe, together with (right) Ca2+ time-series for a select number of neurons. Mean ± 1 SEM.

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